

Effect of Molecular Weight and Method of Deesterification on the Gelling Behavior of Pectin²

BY RUDOLPH SPEISER AND C. R. EDDY

Introduction

The objective of the research described in this paper and the preceding ones^{3,4,5} has been to find an explanation for the marked difference in gelling behavior between pectinic acids deesterified by acid and by enzyme catalysis.

The gelling properties of pectin, as of any high polymer, depend upon molecular weight and type of molecular surface. The latter can be readily ascertained from the degree of esterification³ and acid behavior,⁴ but the determination of molecular

weight presents difficulties because of the complex colloidal behavior of pectin in water solution.

To avoid these complications, Schneider and co-workers^{6,7,8} nitrated pectin and measured molecular weights in acetone solution, a procedure analogous to that commonly used in cellulose chemistry. They also made parallel measurements on pectin acetates from the same samples and obtained viscometric molecular weights agreeing with those of the nitrates. We therefore conclude that the viscometric molecular weight of nitrated pectin in acetone solution is a property of the size of the molecule and not of its surface. This is in contrast to the behavior of pectin in water, where the surface of the molecule is one of the controlling factors in determining viscosity.⁹

(1) One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, United States Department of Agriculture. Article not copyrighted.

(2) Portions of this paper were included in "Factors Influencing the Behavior of Pectinate Gels," by C. H. Hills, H. H. Mottern, G. C. Nutting and R. Speiser, presented at the meeting of the Division of Agricultural and Food Chemistry, American Chemical Society, held in New York, N. Y., September 13, 1944.

(3) C. H. Hills and R. Speiser, *Science*, in press.

(4) R. Speiser, C. H. Hills and C. R. Eddy, *J. Phys. Chem.*, **49**, 328 (1945).

(5) R. Speiser, C. R. Eddy and C. H. Hills, *ibid.*, in press.

(6) F. A. Henglein and G. Schneider, *Ber.*, **69B**, 309 (1936).

(7) G. Schneider and M. Ziervogel, *ibid.*, **69B**, 2530 (1936).

(8) G. Schneider and U. Fritschi, *ibid.*, **69B**, 2537 (1936).

(9) G. L. Baker and M. W. Goodwin, *Delaware Agr. Expt. Sta. Bull.*, 234 (1941); H. S. Owens, H. Lotzkar, R. C. Merrill and M. Peterson, *THIS JOURNAL*, **66**, 1178 (1944). Also see reference included in footnote 2 and Fig. 11 of ref. 5.

Although some degradation occurs during nitration, it is not great, and significant comparisons can be made between samples.

In this paper, we present data on molecular weights of pectin nitrate, from viscosity in acetone solution, and molecular weight distributions obtained through fractionation of the pectin nitrate. These data and information on the type of colloid surface (as indicated by the degree of esterification) are correlated with the gelling properties of the same samples.

Experimental

Source of Materials.—Samples of pectins of high and low degree of esterification were prepared from apple pomace by methods described in preceding papers.^{4,5,10}

Nitration.—A modified Schneider procedure⁹ was used. Five grams of dry apple pectin was nitrated with 250 g. of red fuming nitric acid (sp. gr. 1.54) for one hour at 0° with vigorous stirring. The resulting viscous solution was expelled from a 100-cc. glass hypodermic syringe into 5 liters of distilled water, with the syringe nozzle (without its needle) about 1 cm. above the surface of the water. The water was kept in rapid motion by a stirrer blade designed to circulate as well as stir. Pectin nitrate could be thus precipitated in fibrous form without trapping nitric acid within the aggregates. The precipitate was filtered on a Buchner funnel, washed free of nitric acid, dehydrated by washing with alcohol and dried in a vacuum oven at 40°. In certain cases (H84, H84D, H89C) the product was further purified by dissolving in acetone and reprecipitating with water.

Pectin nitrate obtained by this procedure was fibrous and felt-like when dry, with a yield of 90–95% of theoretical. The nitrogen content ranged from 9.3 to 9.5%, corresponding to 1.6 to 1.8 nitrate groups per galacturonide residue. The ash content was less than 0.1%. Degradation was not great, as shown by Table I. Thus pectin

TABLE I

MOLECULAR WEIGHT OF PECTIN NITRATE AS A FUNCTION OF TIME OF NITRATION AT 0° BY RED FUMING NITRIC ACID (SP. GR. 1.54)

Time of nitration, hours	Molecular weight (from viscosity)
0.75	105,000
1.00	104,000
1.50	99,800
2.00	99,300
0 (Extrapolation)	109,000

nitrated for only one hour, and samples obtained by fractionation of the nitrate, give a reasonable representation of the original pectin sample.

Fractionation.—One to two grams of dry pectin nitrate in 100 cc. of acetone was titrated with toluene at room temperature until the volume of the precipitate, estimated by eye, was approximately the desired fraction of the volume of the original sample. This usually required 1–5 cc. of toluene beyond the cloud point. The precipitate was separated quantitatively by centrifugation, and the liquid was further titrated with toluene to obtain other fractions. The last fraction was quantitatively separated from the solvent mixture by evaporation to dryness. The combined weight of all fractions was within 0.1% of the weight of the original.

In using this method of fractionation, the solvents and the pectin nitrate should be reasonably dry. Otherwise, powdery precipitates difficult to handle are formed. A. C. S. grades of toluene and acetone are satisfactory.

(10) C. H. Hills, J. W. White, Jr., and G. L. Baker, *Proc. Inst. Food Tech.*, 47 (1942).

An attempt was made to fractionate pectin nitrate by titration of acetone solutions with ethyl alcohol. However, samples with low degree of esterification frequently formed either no precipitate or very gelatinous precipitates. Toluene was found to be satisfactory for pectin nitrates of all degrees of esterification.

Measurement of Viscosity.—Viscosities of acetone solutions of pectin nitrate were determined in Ostwald-Fenske capillary pipets at 30.50 ± 0.05°. For each sample, solutions of several concentrations were used having specific viscosities between 0.1 and 0.9.

Calculation of Molecular Weight.—Intrinsic viscosity was obtained by extrapolation of η_{sp}/c versus c to $c = 0$. From this, molecular weight was calculated by the method of Staudinger.¹¹ The Staudinger constant was calculated from data of Schneider and Fritsch⁸ on intrinsic viscosities and osmotic pressures of fractions of a sample of pectin nitrate.

Number-average and weight-average molecular weights for a given sample were calculated from the molecular weights of its individual fractions, on the assumption that each fraction was substantially homogeneous. The usual formulas were used.¹²

Preparation of Gels.—Sixty-five per cent. sugar gels and 35% sugar calcium pectinate gels were made according to the method of Hills, White and Baker.¹⁰ For each sample a series of sugar gels was prepared at several different pH's, and the peak of the curve of gel strength versus pH was taken as the "optimum" gel strength. This procedure is necessary to obtain a gel strength value that is a property of the pectin sample itself and not of incidental factors entering into the preparation of the gels. Likewise, in making calcium gels, a series of different calcium chloride concentrations was used, and that gel was selected which had the highest strength as a function of calcium content.

Determination of Gel Strength.—The Tarr-Baker Delaware Jelly Tester¹³ was employed, and the procedure of Hills, White and Baker¹⁰ was followed. The gel strength is expressed as cm. of water pressure.

Results and Discussion

Viscosity Behavior.—Figure 1 shows extrapolation of η_{sp}/c versus c for a typical series of pectin nitrate fractions. Table II lists the intrinsic viscosities and molecular weights calculated

TABLE II

INTRINSIC VISCOSITIES AND MOLECULAR WEIGHTS OF FRACTIONATED PECTIN NITRATE IN ACETONE

Sample E35, non-deesterified; $\lambda = 0.75$ before nitration

Wt. of fraction (%)	$[\eta]$	Mol. wt.	
34.33	125.7	213,000	
8.75	138.4	235,000	Obs. $\bar{M}_w = 129,000$
6.42	116.9	198,000	Calcd. $\bar{M}_w = 131,000$
5.66	107.4	182,000	Calcd. $\bar{M}_n = 60,200$
44.85	18.9	32,100	

culated from the intercepts of Fig. 1. Degree of esterification (λ) given in all tables is previous to nitration. Nitration produces an approximately 30% decrease in degree of esterification.

The close agreement between observed viscosity-average molecular weight and the weight-average calculated by combining the fractions implies that the exponent a in Flory's equations

(11) H. Staudinger, "Die hochmolekularen organischen Verbindungen," Julius Springer, 1932, also photolithographed by Edwards Bros., 1943.

(12) E. O. Kraemer and W. D. Lansing, *THIS JOURNAL*, 57, 1369 (1935).

(13) L. W. Tarr, *Delaware Agr. Expt. Sta. Bull.*, 142 (1926).

5' and 6 is unity.¹⁴ Analysis of the data of Schneider and Fritsch⁸ according to Flory's equation 5', instead of the simple Staudinger proportionality, also gives a value of α that is equal to unity within the experimental error.

TABLE III

MOLECULAR WEIGHTS OF FRACTIONATED PECTIN NITRATES: EFFECT OF TIME OF DEESTERIFICATION

Sample H91, non-deesterified, $\lambda = 0.74$ before nitration

Wt. of fraction (%)	$[\eta]$	Mol. wt.	
24.68	158.4	268,000	
28.95	67.6	115,000	Obs. $\bar{M}_w = 125,000$
8.24	59.6	101,000	Calcd. $\bar{M}_w = 125,000$
11.24	45.8	77,500	Calcd. $\bar{M}_n = 61,400$
7.28	37.4	63,400	
19.58	12.3	20,800	

Sample H91C, acid deesterified for 18 hours at 40° by 0.9 N HCl, $\lambda = 0.35$ before nitration

22.28	85.6	145,000	
15.96	62.1	105,000	Obs. $\bar{M}_w = 92,400$
9.07	61.0	103,000	Calcd. $\bar{M}_w = 87,400$
6.95	65.8	112,000	Calcd. $\bar{M}_n = 66,300$
4.36	57.0	96,500	
41.37	24.2	41,000	

Sample H91D, acid deesterified for 29 hours at 40° by 0.9 N HCl, $\lambda = 0.24$ before nitration.

7.50	54.0	91,500	
39.68	66.6	113,000	Obs. $\bar{M}_w = 84,000$
7.95	62.3	106,000	Calcd. $\bar{M}_w = 83,500$
6.00	52.8	89,400	Calcd. $\bar{M}_n = 68,600$
7.54	43.6	73,800	
31.33	23.7	40,200	

TABLE IV

MOLECULAR WEIGHTS OF FRACTIONATED PECTIN NITRATES: EFFECT OF METHOD OF DEESTERIFICATION

Sample H84, non-deesterified, $\lambda = 0.80$ before nitration

Wt. of fraction (%)	$[\eta]$	Mol. wt.	
40.36	77.6	131,000	
17.11	64.5	109,000	Obs. $\bar{M}_w = 117,000$
9.06	93.6	159,000	Calcd. $\bar{M}_w = 105,000$
8.73	55.1	93,400	Calcd. $\bar{M}_n = 83,400$
24.72	25.0	42,300	

Sample H84D, acid deesterified for 12 hours at 50° by 0.9 N HCl, $\lambda = 0.32$ before nitration

38.28	68.7	116,000	
12.71	59.3	100,000	Obs. $\bar{M}_w = 86,300$
7.91	69.0	117,000	Calcd. $\bar{M}_w = 80,800$
11.11	26.6	45,100	Calcd. $\bar{M}_n = 57,200$
29.97	18.1	30,700	

Sample H89C, enzyme deesterified for 22.5 minutes at 30° by tomato pectase, $\lambda = 0.35$ before nitration

40.64	80.1	136,000	
16.46	74.2	126,000	Obs. $\bar{M}_w = 102,000$
9.47	65.8	112,000	Calcd. $\bar{M}_w = 102,000$
11.22	42.8	72,600	Calcd. $\bar{M}_n = 76,700$
22.18	20.7	35,100	

(14) P. J. Flory, *THIS JOURNAL*, **65**, 372 (1943).

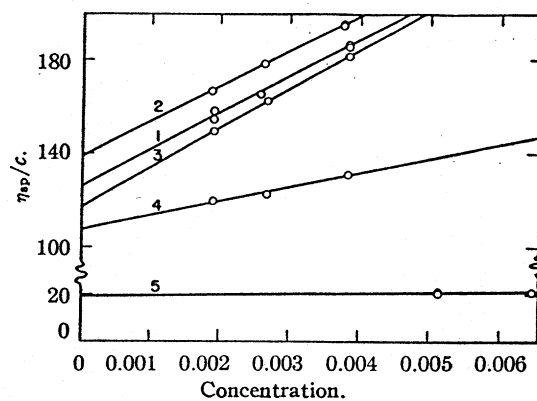


Fig. 1.—Specific viscosity divided by concentration, plotted against concentration, for fractions of pectin nitrate Sample E35 in acetone solution. Concentrations are expressed as moles of galacturonide residues per liter (i. e., grams per liter divided by 260). Numbers indicate order in which the fractions were precipitated.

Similar agreement between observed and calculated weight-average molecular weights is shown by the data in Tables III, IV and V for other samples prepared by various treatments.

TABLE V

MOLECULAR WEIGHTS OF FRACTIONATED PECTIN NITRATES: EFFECT OF PECTINASE DEGRADATION

Sample H59, acid deesterified for 18 hours at 50° by 1 N HCl, $\text{CH}_3\text{O} = 4.53\%$ before nitration

Wt. of fraction (%)	$[\eta]$	Mol. wt.	
34.57	156.3	265,000	
20.33	127.4	216,000	Obs. $\bar{M}_w = 195,000$
8.96	155.8	264,000	Calcd. $\bar{M}_w = 199,000$
13.09	117.0	198,000	Calcd. $\bar{M}_n = 143,000$
23.03	36.4	61,700	

Sample H74, enzyme deesterified for 98 minutes at 50° by a mixture of pectase and diastase, contaminated with a small amount of pectinase, $\text{CH}_3\text{O} = 4.48\%$ before nitration

33.78	154.4	262,000	
30.20	99.2	168,000	Obs. $\bar{M}_w = 154,000$
16.06	64.4	109,000	Calcd. $\bar{M}_w = 162,000$
6.81	35.3	59,700	Calcd. $\bar{M}_n = 64,400$
13.14	7.9	13,400	

From the slopes of graphs of η_{sp}/c versus c values of the Huggins¹⁵ constant k' have been calculated for two pectin nitrate samples of different degree of esterification and are presented in Table VI. With the exception of the first fraction of H91D, k' is approximately constant for the fractions of a given sample but different for the two samples of different history. By applying the theories of Frith¹⁶ to the equations of Huggins¹⁵ it can be shown¹⁷ that k' should be higher the greater the energy of interaction be-

(15) M. L. Huggins, *ibid.*, **64**, 2716 (1942).

(16) E. M. Frith, *Trans. Faraday Soc.*, **41**, 17, 90 (1945); E. M. Frith and R. F. Tuckett, *Nature*, **155**, 164 (1945).

(17) R. Speiser and R. T. Whittenberger, *J. Chem. Phys.*, **13**, 349 (1945).

TABLE VI
HUGGINS CONSTANT, $k' = \frac{1}{[\eta]^2} \frac{d(\eta_{sp}/c)}{dc}$, FOR TWO FRACTIONATED PECTIN NITRATES IN ACETONE

Fraction	Sample E35 $\lambda = 0.75^a$ k'	Sample H91D $\lambda = 0.24^a$ k'
1	0.99	2.10
2	0.79	0.15
3	1.23	.06
4	0.52	.27
5	0.78	.03
6	..	.32
Average ^b	0.87 ± 0.08	$.19^c \pm 0.05$

^a Previous to nitration. ^b Calculated from formulas of Birge,^d weighted according to the amount of material in each fraction. The low value of the probable error is due to the fact that the large deviations are associated with the smaller fractions. ^c Omitting first fraction (see text). ^d R. T. Birge, *Phys. Rev.*, 40, 207 (1932).

tween solvent and solute. Since acetone should have a greater attraction for a methyl ester group than for a carboxyl group, k' would therefore be expected to be greater for Sample E35 (75% esterified) than for Sample H91D (24% esterified) which is confirmed in Table VI.

As mentioned under "Experimental," this dependence of k' upon degree of esterification is also reflected in the precipitation behavior. Ethanol is a satisfactory fractional precipitant for a pectin nitrate of high degree of esterification, but a pectin nitrate of low degree of esterification forms either a gel or no precipitate, because ethanol is more compatible with free carboxyl groups than with methyl ester groups (possibly due to hydrogen bonding). On the other hand, toluene is sufficiently non-polar to be incompatible with both carboxyl groups and methyl ester groups and will therefore satisfactorily precipitate pectin nitrates of all degrees of esterification from acetone solution.

We have found that in fractional precipitation of pectin nitrate the first fraction is often not the one of highest molecular weight and also sometimes has a value of k' that is considerably differ-

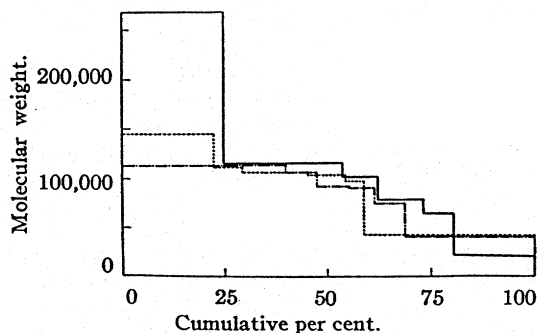


Fig. 2.—Molecular-weight distribution of pectin nitrate as a function of time of acid deesterification at 40°: —sample H91, non-deesterified; ----sample H91C, deesterified for 18 hours; —.—sample H91D, deesterified for 29 hours.

ent from that of the other fractions. Similar behavior has been reported for cellulose acetate and other polymers.¹⁸ The molecules of this fraction may be supposed to differ somewhat from the remaining fractions in their surface structure, such as in average nitrate or methyl content. However, this anomaly of the first fraction apparently does not affect the validity of the extrapolated $[\eta]$ and hence of the molecular weight, since calculated and observed weight-average molecular weights are always self-consistent.

Molecular Weight and its Distribution.—The data of Tables III, IV and V are assembled in histogram forms in Figs. 2, 3 and 4. From these diagrams the distribution in molecular weight of each sample can be visualized, and from the total area under each curve the weight-average molecular weight can be obtained. The difference between weight- and number-average molecular weights is also a measure of the homo-

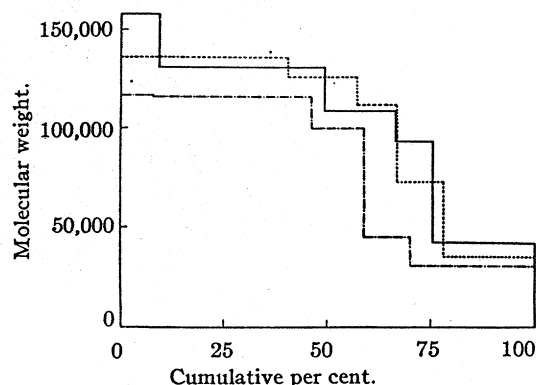


Fig. 3.—Molecular-weight distribution of pectin nitrate as a function of method of deesterification: — sample H84, non-deesterified; ---- sample H89C, enzyme deesterified for 22.5 minutes at 30°; —.— sample H84D, acid deesterified for twelve hours at 50°.

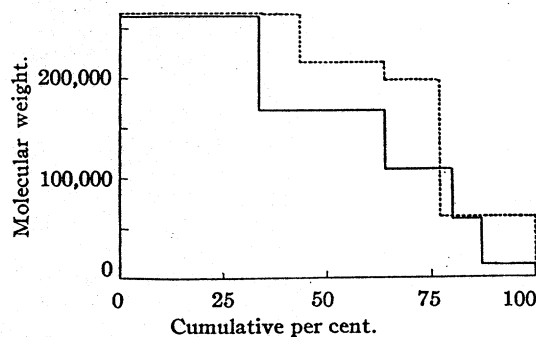


Fig. 4.—Molecular-weight distribution of pectin nitrate. Effect of pectinase degradation: ---- sample H59, acid deesterified for 18 hours at 50°; — sample H74, deesterified for 98 minutes at 50° by a pectase-diastrase mixture contaminated with a small amount of pectinase.

(18) H. M. Spurlin, *Ind. Eng. Chem.*, 30, 538 (1938); A. M. Sookne, H. A. Rutherford, H. Mark and M. Harris, *J. Res. Natl. Bur. Standards*, 29, 123 (1942).

geneity of the sample; the closer the weight and number averages approach equality, the more homogeneous the sample.

Figure 2 shows the change in molecular weight distribution with time of acid deesterification. Although the number-average molecular weight appears not to change very much, this constancy is only fortuitous because of loss of low molecular weight material during the purification that followed deesterification. The weight-average molecular weight is, on the other hand, insensitive to variations in the low molecular-weight fractions and hence is a better measure of the amount of degradation than the number-average molecular weight.

It can be seen from Fig. 2 that the weight-average molecular weight drops rapidly during the early part of the reaction, suggesting on cursory examination that extensive degradation has occurred. This drop in molecular weight is also reflected in the viscosity behavior in aqueous solution. One per cent. solutions of Samples H91, H91C, and H91D had viscosities in the ratio 1:0.45:0.42 at 19.8°; 1.2% solutions of Samples H84 and H84D had viscosities in the ratio 1:0.38 at 30.5°.

It has been suggested¹⁹ that the initial rapid fall in viscosity of pectin solutions on heating, with only a small increase in the number of reducing groups, is due to the presence in pectin of more than one type of polymer linkage. However, equation 15 and Fig. 1 of Montroll and Simha²⁰ show that one expects theoretically a sharp drop in weight-average molecular weight during the early stages of polymer degradation, although only a very small fraction of the bonds are broken, whereas in the later stages of the reaction a much greater fraction of the bonds must be broken to produce the same drop in weight-average molecular weight. For Samples H91C and H91D it can be calculated from the equation of Montroll and Simha that only 0.23 and 0.29%, respectively, of the bonds need be broken to give the observed drop in molecular weight. Likewise only 0.22% of the bonds need be broken to give the observed molecular weight of Sample H84D. Thus the observed behavior is predicted theoretically for a uniform molecule, without the necessity of assuming that weak linkages break during the early part of the reaction and stronger linkages during the later part.

Figure 3 shows the difference between the effects of acid and enzyme deesterification on the molecular weight distribution. Acid deesterification leads to considerably more degradation and heterogeneity than does enzyme deesterification when pectinase action is minimized.⁶

Figure 4 contrasts a high molecular weight acid-deesterified pectin with another high molecular weight pectin deesterified by a mixture

of pectase,²¹ diastase and pectinase²¹ under conditions where pectinase action was not minimized. This type of enzyme treatment led to a heterogeneous product, with a considerable amount of low molecular weight material in spite of its high weight-average molecular weight. In this case the acid-treated sample was much more homogeneous.

Gelation.—In our studies we have used two types of gels: (1) a small amount of pectin in a 65% aqueous sucrose solution ("sugar gel"), and (2) small amounts of pectin and calcium salt in a 35% aqueous sucrose solution ("calcium gel"). These two types of gels are fundamentally different in the manner of cross linking of the pectin molecules into a three-dimensional structure. The cross links in the sugar gels are all hydrogen bonds, the sugar functioning as a hydrogen bonding agent. The cross links in the calcium gels are partly hydrogen bonds and partly ionic calcium bonds between pairs of carboxyl groups in adjacent molecules.

Since the strength of a sugar gel is strongly dependent on the pH, with a maximum at a particular pH value, and since the pH corresponding to this maximum varies with degree of esterification,^{10,22,23} it is necessary to select the pH that will give the maximum gel strength of each sample, in order to obtain a significant measure of the intrinsic gelling power of the sample. Applying a single fixed pH to all samples of different degrees of esterification would give gel strength values that have no general significance. For this reason, the "optimum gel strengths" reported in this paper are maxima obtained from curves of gel strength *versus* pH.

TABLE VII
GEL STRENGTHS OF VARIOUS PECTINS

Sample	% CH ₃ O	Degree of esterification ^a	Calcd. \bar{M}_w	Calcd. \bar{M}_n	Optimum gel strength	
					65% sugar gels, cm.	35% Ca pectinate gels, cm.
H91	9.44	0.74	125,000	61,400	20	0
H91C ^a	5.13	.35	87,400	66,300	0	87
H91D ^a	3.73	.24	83,500	68,600	0	85
H84	8.88	.80	105,000	83,400	61	0
H84D ^a	4.72	.32	80,800	57,200	20	30
H89C ^b	4.80	.35	102,000	76,700	47	13
H59 ^a	4.53	..	199,000	143,000	72	56
H74 ^b	4.48	..	162,000	64,400	24	4

^a Acid deesterified. ^b Enzyme deesterified.

(21) The nomenclature used here is that of the Committee on Nomenclature of Pectin, *THIS JOURNAL* (Proceedings), **49**, 37 (1927).

(22) P. B. Myers and G. L. Baker, *Delaware Agr. Expt. Sta. Bull.*, 149 (1927); G. L. Baker and M. W. Goodwin, *ibid.*, 234 (1941); *ibid.*, 246 (1944); also see reference included in footnote 2.

(23) In the presence of small amounts of polyvalent cations, such as calcium, magnesium or aluminum, this pH optimum is in some cases wiped out. However, the strength of the ionic bond is so great in comparison to the hydrogen bond that polyvalent cations must be rigorously excluded before conclusions can be drawn on the properties of hydrogen bonded gels.

(19) Z. I. Kertesz, *THIS JOURNAL*, **61**, 2544 (1939).

(20) E. W. Montroll and R. Simha, *J. Chem. Phys.*, **8**, 721 (1940).

For calcium gels, the gel strength is also sharply dependent upon calcium concentration.¹⁰ Therefore, the optimum gel strength for these ionic bonded gels must be the maximum obtained by varying calcium content.

Table VII gives some representative data, selected from a large number of measurements, correlating gel strength with molecular weight and degree of esterification. Within a given series, the strengths of the sugar gels are determined largely by weight-average molecular weight substantially independent of degree of esterification. Apparent deviations from this rule, when different series are compared, are due to factors difficult to control, such as solubility, ballast²⁴ content and ash content.

The strengths of the calcium gels, on the other hand, depend on degree of esterification. As can be seen from Samples H84 and H91 and from previous publications,^{2,3,10} pectins of high degree of esterification will not form calcium gels, even though they will form sugar gels. However, pectins of too low molecular weight to form sugar gels (H91C, H91D) will sometimes form strong calcium gels if the degree of esterification is low. This is because the ionic bond is considerably stronger than the hydrogen bond and hence is more capable of making up for the smaller number of main-chain bonds.

Samples H84D and H89C show that degree of esterification and molecular weight are not the only factors controlling calcium gel strength. Both H84D and H89C were prepared from the same starting material and have approximately the same degree of esterification. H89C has the higher molecular weight, the greater homogeneity and the higher 65% sugar gel strength. Nevertheless the H89C calcium gel is less than half as strong as that of H84D. Similarly, the sugar gel strengths of H59 and H74 are in the ratio of 3:1, whereas their calcium gel strengths are in the ratio of 14:1. This behavior is due to a fundamental difference in the mechanisms of acid and enzyme demethylations and has been observed repeatedly in this Laboratory.¹⁰

An explanation for this discrepancy between sugar gels and calcium gels from enzyme-deesterified pectins can be found in the following two facts: (1) The amount of calcium required for maximum gel strength decreases as the degree of esterification decreases.^{2,10} (2) The degree of esterification of a sample is an average summed over all molecules and will follow a continuous distribution. One would expect that, because of the randomness of acid deesterification, all molecules in a sample would be demethylated to

about the same extent, so that each molecule would have a degree of esterification not greatly different from the average. On the other hand, one would expect that the greater selectivity in the action of an enzyme might result in the demethylation of certain molecules much more than others. Thus an enzyme-deesterified sample might contain a substantial fraction of the molecules with low degree of esterification and another substantial fraction with high degree of esterification, although the average for the whole sample might be intermediate in value.

In making a calcium gel from an enzyme-deesterified sample, it would therefore be impossible to select a calcium concentration that would be optimum for all the molecules. A calcium content which is optimum for the molecules of low degree of esterification would not be sufficient to form strong bonds between the molecules of high degree of esterification, and a calcium content high enough to be optimum for the molecules of high degree of esterification would produce incipient precipitation of the molecules of low degree of esterification. Since the curve of gel strength *versus* calcium concentration is sharply peaked, any calcium concentration selected would link effectively only a portion of the pectin molecules in the sample. On the other hand, a single calcium concentration is optimum for nearly all the molecules of an acid-deesterified sample. Therefore, if all other factors are equal, a calcium gel made from an acid-deesterified pectin of intermediate degree of esterification should be stronger than one made from an enzyme-deesterified pectin of the same average degree of esterification. Electrophoresis data supporting this hypothesis will be given in a later paper.

Acknowledgment.—We wish to acknowledge the valuable assistance of Mrs. Evelyn E. Karr in performing much of the experimental work.

Summary

1. Molecular weight and molecular weight distribution have been determined for pectins deesterified by acid and enzyme catalysis. Viscosity of nitrated pectin in acetone solution was used for determining the molecular weight. Pectin nitrate was fractionally precipitated from acetone solution by toluene for determination of the molecular weight distribution.

2. Gelling behavior of the pectin samples (before nitration) has been correlated with molecular weight, degree of esterification and method of deesterification. The strengths of hydrogen-bonded gels (65% sucrose gels) are primarily determined by molecular weight, gel strength increasing with increasing molecular weight, substantially independent of degree of esterification and method of deesterification.

3. The strengths of ionic-bonded gels (35% sucrose calcium pectinate gels) are less affected by molecular weight than hydrogen-bonded gels

(24) For the purposes of this series of papers, "ballast" is defined as any non-uronide organic material in a sample of pectin whether or not attached to the polygalacturonide chain, in general agreement with Olsen²⁵ and Schneider.²⁶ The major constituents of ballast are galactan and araban.

(25) A. G. Olsen, R. F. Stuewer, E. R. Fehlberg and N. M. Beach, *Ind. Eng. Chem.*, **31**, 1015 (1939).

(26) G. G. Schneider and H. Bock, *Ber.*, **70B**, 1617 (1937).

but are strongly dependent on degree of esterification, being highest for degree of esterification in the range 0.3 to 0.5. Enzyme-deesterified pectins

form weaker ionic-bonded gels than do acid-deesterified pectins.
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